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CONTENTS

PAGE.

Observations on the Bactericidal Efficiency of Single-unit Vacreators. By L. L. MULLER 19

The Value of the Resazurin Test for the Determination of Milk Quality. By W. F. SCHUBERT 29

A Comparison of a Quaternary Ammonium Compound and a Calcium Hypochlorite Compound as a Germicidal Agent. By W. F. SCHUBERT 36

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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

Vol. 8. - - Nos. 2 & 3.

JUNE and SEPTEMBER, 1951.

OBSERVATIONS ON THE BACTERICIDAL EFFICIENCY OF SINGLE-UNIT VACREATORS.

By L. L. MULLER, B.Sc., Assistant Bacteriologist, Dairy Research Laboratory, Division of Dairying.

SUMMARY.

Bactericidal efficiency appears to reach a critical level at a pasteurizing temperature of about 200° F. A considerable loss in efficiency occurs for each degree below about 200°.

The bacteriological quality of the raw cream has an appreciable influence on the survival of thermoduric bacteria.

Misuse of the equilibrium valve, rate of cream flow, and intensity of cream treatment have no apparent influence on the bacteriological quality of the treated cream.

A method for the laboratory pasteurization of ripened cream is suggested.

INTRODUCTION.

In most Queensland butter factories a vacreator is used to pasteurize and deodorize the soured cream supplied for manufacture into butter. The Department's Butter Improvement Service (Muller and Nichols, 1950) commonly showed high plate counts in butters from some of the factories equipped with single unit vacreators, and very high bacteria counts were frequent in freshly pasteurized cream examined during surveys of these factories. While there was no reason to suspect that the types of bacteria surviving pasteurization in this manner were harmful to cream or butter quality, difficulties in the fair assessment of Butter Improvement Service and factory survey results made it desirable to find the cause of this high survival of bacteria. For this reason, experiments were conducted to investigate the factors in the operation of a single unit vacreator which would be liable to influence its bactericidal efficiency.

These factors include:—

(1) misuse of the equilibrium valve;

(2) the pasteurizing temperature;

(3) the bacteriological quality of the raw cream;

(4) the rate of cream flow; and

(5) the intensity of cream treatment.

PROCEDURE.

The single unit vacreators employed in the experimental work were of standard design, consisting of the following parts:—

(1) A pasteurizing chamber in which cream is mixed with steam to reach a pasteurizing temperature corresponding to the boiling point of water at the partial vacuum existing in the chamber.

(2) A deodorizing chamber in which the hot cream is subjected to a higher vacuum and in consequence boils vigorously. The vacuum difference between the two chambers is controlled by the equilibrium valve.

(3) An ejector-condenser which creates the vacuum by means of a jet of water and condenses the vapour evolved during boiling of the cream in the deodorizing chamber.

The method adopted was to treat a vat of cream, graded choice by platform examination, under conditions as standard as could be obtained, except for alterations in one variable. Single plate counts were done on duplicate samples of pasteurized cream taken at intervals, and all plates of laboratory pasteurized cream were made by single plates on triplicate samples, pasteurized in triplicate. Plate counting methods similar to those described by Muller and Nichols (1950) were used.

The accuracy of the plate counting methods may be summarized as follows. In 65 sets of counts on duplicate or triplicate samples, the mean variation from the mean of the counts was $\pm 11{\cdot}1$ per cent. One pair showed a variation of ± 73 per cent. from their mean count, six were between 20 and 28 per cent. and the remainder were within ± 0 to 19 per cent. of their mean. It is considered that this degree of inaccuracy did not materially affect the significance of the findings.

RESULTS.

The Influence of the Equilibrium Valve.

During the operation of a vacreator the pasteurizing temperature should be largely controlled by raising or lowering the partial vacuum in the pasteurizing chamber by means of adjustments in the tension of the equilibrium valve. Observations in factories had frequently shown that operators did not appreciate this point and were inclined to leave the valve slack or even remove it to hasten the cream flow. On the assumption that the equilibrium valve would slightly delay passage of individual cream particles, it was considered possible that a variation sufficient to increase the bacterial survival may occur in the time factor of the temperature-time relationship to bacterial destruction.

An experiment was designed to treat one half of a vat of cream without the equilibrium valve and the remaining half with the valve operating and as

near as practicable to the same conditions of vacuum, temperature and rate of cream flow. The experiment was performed on two successive days with results as in Table 1.

Table 1.

Sample No.	Pasteurizing Temp. (°F.)	Equivalent Vacuum (in.)	Deodorizer Vacuum (in.)	Mean Plate Count per ml.
		TRIAL A.		
		EQUILIBRIUM VALVE OUT.		
1 ..	184	13	13	3,000,000
2 ..	200	6½	13	120,000
3 ..	202	5½	12½	120,000
4 ..	202	5½	13	290,000
		EQUILIBRIUM VALVE IN.		
5 ..	203	5	15	130,000
6 ..	201	6	15	220,000
7 ..	200	6½	15	420,000
8 ..	200	6½	15	650,000
		TRIAL B.		
		EQUILIBRIUM VALVE OUT.		
9 ..	196	8½	15	1,950,000
10 ..	196	8½	15	2,250,000
11 ..	196	8½	15	1,800,000
		EQUILIBRIUM VALVE IN.		
12 ..	202	5½	15	660,000
13 ..	201	6	15	680,000
14 ..	201	6	15	700,000

Practical difficulties in the operation of the vacreator prevented the attainment of the same conditions of temperature and vacuum with the valve in and out; the variability was greater in trial A than in trial B. However, examination of the results suggested that the variations in count in each trial could be ascribed to the changes in pasteurizing temperature. Again, the difference between the counts obtained in trials A and B at similar temperatures possibly could be related to the difference in bacteriological quality of the raw cream on the two days. The results showed no evidence that the postulated variation in the time factor of the temperature-time relationship to bacterial destruction operated.

The Influence of the Pasteurizing Temperature.

To investigate the significance of changes in the pasteurizing temperature within the range normally used (viz., 195-202 deg. F.), an experiment was designed in which a vat of cream was treated at gradually increasing temperatures while an endeavour was made to keep the vacuum and rate of cream flow the same. This was carried out on three successive days at one factory and on two successive days at another factory to eliminate possible variations between vacreator units. The results are set out in Table 2.

Table 2.

Sample No.	Pasteurizing Temperature (°F.)	Deodorizing Vacuum (in.)	Mean Plate Count per ml.	Mean Plate Count of Lab. Past. Cream.	Percentage of Lab. Past. Count.
			FACTORY A.		
			TRIAL 1.		
1	194	15	2,000,000	16,500,000	12·1
2	196	14½	1,760,000	..	10·7
3	198	14½	1,130,000	..	6·9
4	200	13½	830,000	..	5·0
5	202	14	810,000	..	4·9
6	205	14	600,000	..	3·6
			TRIAL 2.		
1	193·5	16	1,250,000	7,200,000	17·6
2	196	15	850,000	..	11·8
3	198	14½	470,000	..	6·5
4	200	14	410,000	..	5·7
5	202	15	320,000	..	4·5
6	204	15	320,000	..	4·5
			TRIAL 3.		
1	194	14½	500,000	3,900,000	12·8
2	197	15	370,000	..	9·5
3	198	14	270,000 ..	..	6·9
4	200	14	170,000	..	4·4
5	202	14	130,000	..	3·3
6	204	14	90,000	..	2·3
			FACTORY B.		
			TRIAL 4.		
1	194	15	635,000	670,000	94·8
2	196	15	530,000	..	85·5
3	198	15	380,000	..	56·7
4	200	15	375,000	..	56·0
5	202	15	150,000	..	22·4
6	203·5	15	135,000	..	20·2
			TRIAL 5.		
1	194	15	2,100,000	1,810,000	116
2	196	15	1,605,000	..	88·7
3	198	15	820,000	..	45·4
4	200	15	460,000	..	25·4
5	202	15	160,000	..	8·9
6	203·5	15	150,000	..	8·3

These results show that variations in pasteurizing temperature of even two degrees may have an appreciable influence on the bacterial survival.

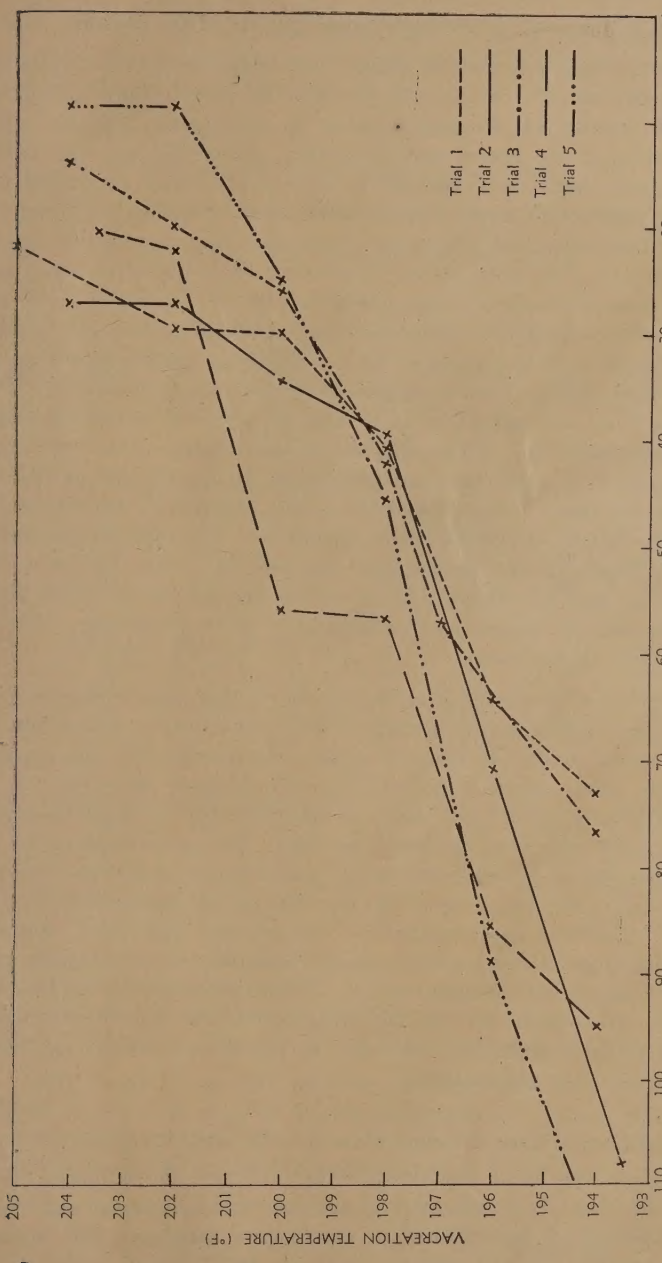

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Figure 1.

Vacreation Temperature and Bactericidal Efficiency.

The Influence of the Bacteriological Quality of the Cream.

The preliminary experiments on the use of the equilibrium valve had shown the possibility of considerable variation in plate counts due to the bacteriological quality of the raw cream. To compensate for this effect, triplicate samples of the cream were laboratory pasteurized and the factory pasteurized cream counts were expressed as a percentage of the counts obtained on laboratory pasteurized cream. In the first three experiments (Trials 1-3) laboratory pasteurization was performed on undiluted raw cream for 35 minutes at 62.5-63.0 deg. C. The plate counts obtained at this temperature were much higher than those obtained on cream treated with the vacreator. For this reason trials were conducted to determine a more suitable method and temperature. This resulted in the adoption of the following technique, which was used for all later trials. Cream samples were taken in triplicate and 1 ml. transferred to 9 ml. of sterile tap water with 0·1 per cent. of agar added to improve the emulsification. Five millilitres of this diluted cream were transferred aseptically to a sterile tube and pasteurized in a water bath at 70.5-71.0 deg. C. for 35 minutes. Table 2 shows the results obtained in this manner for trials 1-5, and Figure 1 shows the results graphically. For the sake of clarity, in compiling Figure 1 the percentages obtained for trials 1-3 have been multiplied by an arbitrary figure six so as to compensate for the much higher laboratory pasteurized counts and consequently lower percentages obtained when 62.5-63 deg. C. was used.

From the close proximity of all the curves relating pasteurizing temperature to the percentages obtained as above, it will be seen that the variability in the bacteriological quality of the raw cream accounts for the discrepancies observed in the plate counts of different batches of cream pasteurized at the same temperature. Figure 1 also shows the marked effect of small changes in pasteurizing temperature on bacterial survival. The general slope of the curves from 194 deg. F. to about 200 deg. F. shows that a considerable improvement in the bacteriological quality of the resulting cream is obtainable for each degree F. rise in pasteurizing temperature. Above about 200 deg. F., however, the slope changes and there is comparatively little improvement in the plate count for each degree rise in temperature. These results appear to show that 200 deg. F. can be taken as a critical temperature for good bacteriological results when using a single-unit vacreator to pasteurize cream containing a high count of heat-resistant bacteria.

The Effect of Rate of Cream Flow and the Intensity Factor.

A further experiment was designed to determine the influence on the bactericidal efficiency of the vacreator of the rate at which cream was treated and of the intensity of deodorization in the vacuum chamber. This intensity is expressed by the "Intensity Factor" (Smith, 1947), which is the amount

of heat (B.T.U.) expended on deodorization per pound of cream treated. The experiment, which was repeated on three successive days, consisted of treating a vat of cream in four stages, namely:—

(1) 195 deg. F. and low rate of cream flow;

(2) 200 deg. F. and low rate of cream flow;

(3) 200 deg. F. and high rate of cream flow; and

(4) 195 deg. F. and high rate of cream flow.

Table 3.

Sample Number.	Past. Temp. (°F.)	Vacuum (in.)	Rise in Temp. of Condenser Water (°F.)	Condenser Water Pressure (lb./sq. in.)	Intensity Factor.	Rate of Cream Flow (galls./hr.)	Mean Plate Count per ml.	Percentage of Lab. Past. Count.
			TRIAL 1.					
1 ..	Laboratory Pasteurized Cream			..	..	..	1,130,000	..
2 ..	195	16½	21	92	61	720	2,020,000	179
3 ..	200	16½	33	92	96	720	490,000	43
4 ..	200	16½	32	92	70	950	600,000	53
5 ..	195	16½	24	92	53	950	1,760,000	156
			TRIAL 2.					
1 ..	Laboratory Pasteurized Cream			..	..	..	1,100,000	..
2 ..	195	15	49	92	205	500	1,410,000	128
3 ..	200	15	56	92	234	500	830,000	75
4 ..	200	15	54	92	141	800	650,000	59
5 ..	195	15	50	92	131	800	1,510,000	138
			TRIAL 3.					
1 ..	Laboratory Pasteurized Cream			..	..	..	1,620,000	..
2 ..	195	15	47	92	164	600	1,600,000	99
3 ..	200	15	49	92	171	600	360,000	22
4 ..	200	15	41	92	101	850	480,000	30
5 ..	195	15	45	92	111	850	1,490,000	92

Again laboratory pasteurization of the raw cream and expression of the plate counts of the vacreated cream as a percentage of the laboratory pasteurized counts was used as a device to eliminate the influence of the bacteriological quality of the cream. The results are shown in Table 3 and Figure 2.

The results show that the rate of cream flow used has no apparent influence on bacterial survival during pasteurization. This is shown in Fig. 2, where the slight variation of the lines from the vertical is apparently at random and can be accounted for by the inaccuracy of the plate count.

The Intensity Factor is given by the expression:—

$$\frac{\text{Gallons of water per hour} \times \text{Temperature rise}}{\text{Gallons of cream per hour}}$$

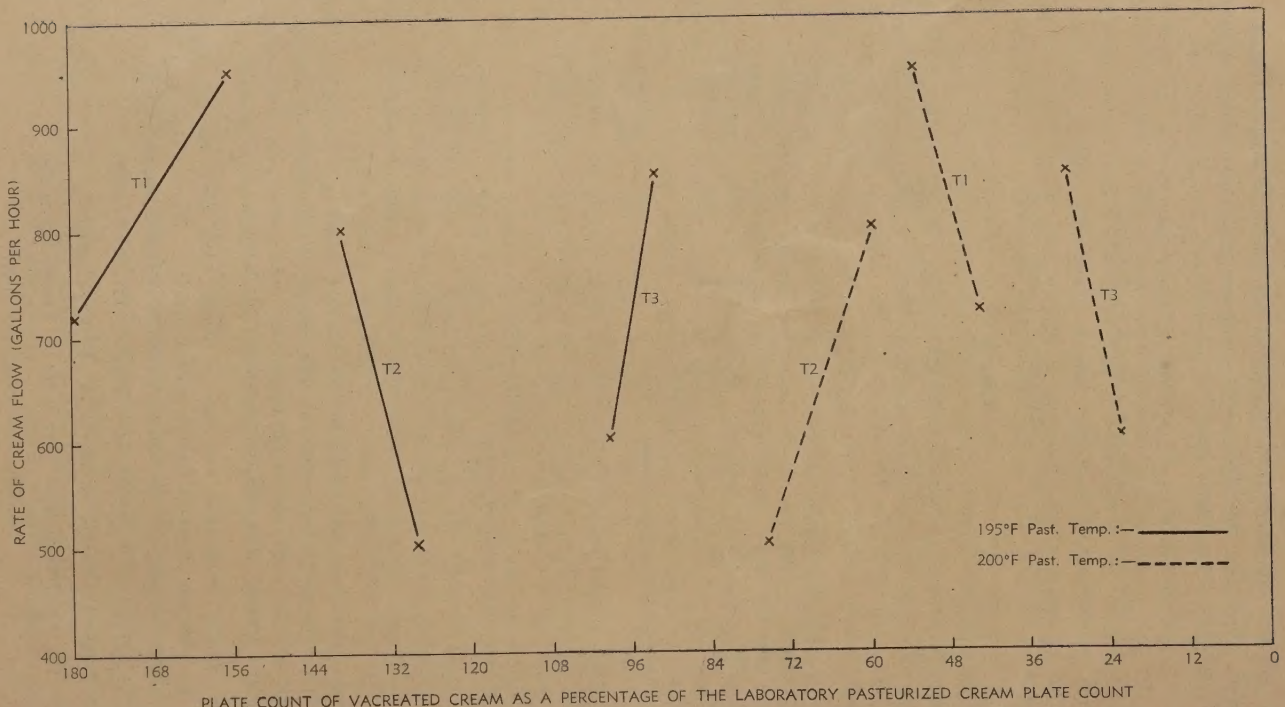

Figure 2.

Rate of Cream Flow and Bactericidal Efficiency.

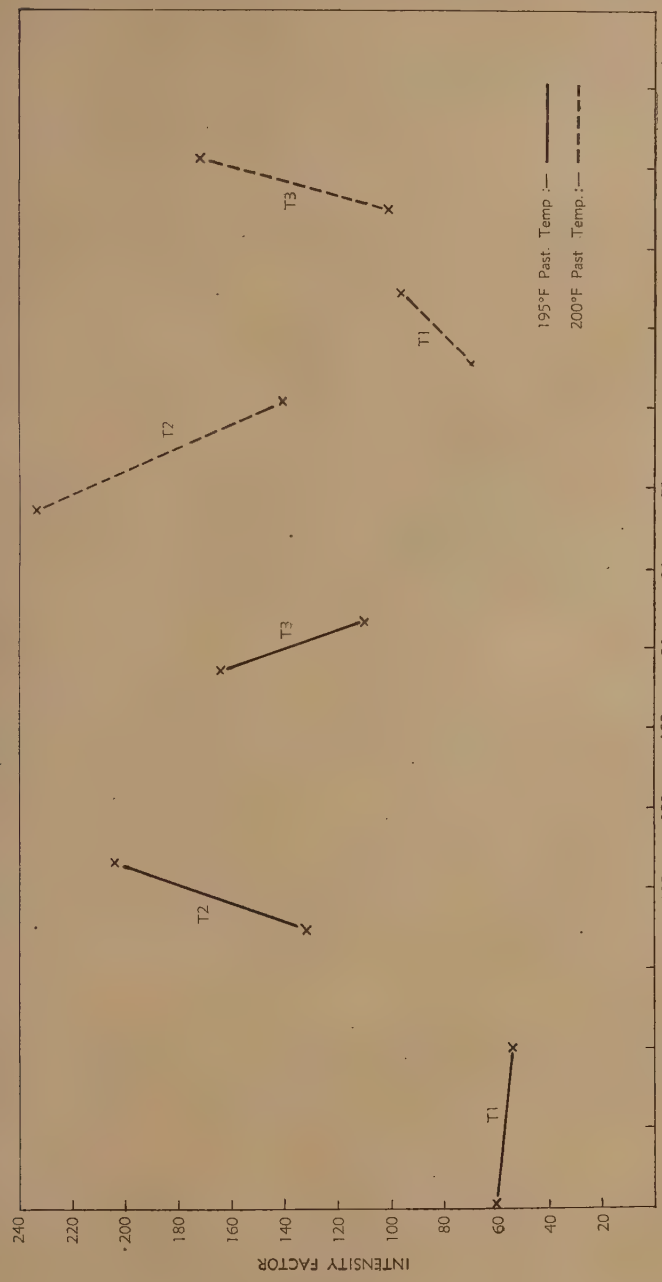

Figure 3.

Intensity Factor and Bactericidal Efficiency.

The number of gallons of water per hour through the condenser is given by the water pressure and would have been 2,090 in these trials. The temperature rise between the incoming and outgoing water of the condenser is given in Table 3 and is fairly constant for corresponding pasteurizing temperatures in each trial. Thus if the figures for 200 deg. F. pasteurizing temperatures are examined it will be seen that, though the intensity factor is altered considerably by the changing rates of cream flow, the change in the plate count of the pasteurized cream is not significant. This is further illustrated in Figure 3, where the variations of the lines from the vertical can be considered as random.

CONCLUSIONS.

The results obtained show that, of the factors considered likely to result in a high survival of thermoduric bacteria during pasteurization in a single unit vacreator, the only two having an appreciable influence are the pasteurizing temperature and the bacteriological quality of the raw cream.

In the case of the pasteurizing temperature, the results also show that a considerable loss in bactericidal efficiency occurs for each degree below about 200 deg. F. and that the corresponding gain in efficiency by pasteurizing above 200 deg. F. is not so great. It would appear that 200 deg.-202 deg. F. could be taken as a temperature range to achieve reasonably good results with a single unit vacreator.

The bacteriological quality of the raw cream is not necessarily related to the grading quality of the cream, all cream used in the experiments being of choice quality. There are no means available in factory practice for selecting cream of good bacteriological quality, so it would appear necessary to rely on the pasteurizing temperature alone to achieve good bacteriological results in pasteurized cream.

While it has been shown that misuse of the equilibrium valve, the rate of cream flow and the intensity of cream treatment have no apparent influence on the bacteriological results, it should be stressed that these three factors are of considerable importance in the second phase of vacreation, namely, the deodorization of cream and consequent improvement in the grading qualities of the resultant butter.

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SMITH, T. W. 1947. Cream vacreation—a simple explanation of the "Intensity Factor." Qld. Agric. J. 65: 143-5.

THE VALUE OF THE RESAZURIN TEST FOR THE DETERMINATION OF MILK QUALITY.

By W. F SCHUBERT, B.A., B.Sc., A.Ed. (Bacteriologist) and PATRICIA M. NAGLE, B.Sc. (Assistant) Dairy Research Branch, Division of Dairying.

SUMMARY.

Both the ten-minute and the one-hour resazurin tests were compared with the "modified" methylene blue test, the standard plate count at 37°C., the direct microscopic count, and the leucocyte count.

Neither of the resazurin tests has any advantage over the "modified" methylene blue test for the examination of milk received at depots.

INTRODUCTION.

The work reported in this paper was prompted by the interest displayed by market milk organizations in the resazurin test and a desire to introduce it as a routine test at milk factories in preference to the "modified" methylene blue test. An effort has been made to determine its value in relation to other standard milk quality examinations on a relatively large and representative number of milk samples.

Most market milk grading programmes combine organoleptic classification with a dye reduction test. Though both the "old" methylene blue test and the "modified" methylene blue test of Wilson (1935) are widely used as indices of bacteriological quality, in recent years there has been a revival of interest in the resazurin test, which was first publicised by Ramsdell, Johnson and Evans (1935). Resazurin was reported by these investigators to be slightly more electro-positive than methylene blue, thus requiring a shorter incubation period. As the time lag involved in the methylene blue test has proved a serious disadvantage, the search for a shorter test has been a major factor in the stimulation of renewed interest in the resazurin test.

MATERIAL AND METHODS.

Three hundred and forty farmers' milks were sampled in the routine way on the receiving platform of a Brisbane milk depot and submitted to:—

(1) The Ten-minute Resazurin Test;

(2) The One-hour Resazurin Test;

(3) The "Modified" Methylene Blue Test;

(4) The Standard Plate Count at 37 deg. C.;

(5) The Direct Microscopic Count; and

(6) The Leucocyte Count.

No controlled ageing of the milk samples in the laboratory was attempted. The average age of samples on testing was approximately three hours, due to the time elapsing between milking of the herd and arrival of the milk at the depot.

The following methods were followed:—

1. Resazurin Test.—The resazurin testing was carried out according to the technique outlined by the Ministry of Agriculture and Fisheries of Great Britain (1943). The dye used was prepared by the National Aniline and Chemical Company Inc. for use as an indicator of reduction in milk and distributed by Eastman Kodak Company, of Rochester, New York State, U.S.A. A 0.05 per cent. stock solution was prepared and kept stored in a refrigerator. This was diluted 1 to 10 as required. A Lovibond flat-type resazurin comparator was used for reading the tests.

2. "Modified" Methylene Blue Test.—The methylene blue testing was carried out according to the modified technique of Wilson (1935). B.D.H. methylene blue tablets as approved by the Ministry of Agriculture and Fisheries of Great Britain were used throughout the tests.

3. Standard Plate Count at 37 deg. C.—The standard plate counts were made on tryptone-glucose-extract-milk agar as recommended by the American Public Health Association (1941), with incubation at 37 deg. C. for 48 hours.

4 Direct Microscopic Counts.—These were made according to the recommendations of the American Public Health Association (1941). Twenty fields were counted.

5. Leucocyte Count.—Twenty fields of each direct microscopic count smear, referred to in (4), were counted.

RESULTS.

The Ten-minute Resazurin Test.

1. Comparison with the "Modified" Methylene Blue Test.

An examination of Table 1 reveals that of the 282 samples giving a "modified" methylene blue test reading of four hours or greater, 280 were covered by resazurin test grades 6 and 5. Of the 58 milks which failed a 4-hour "modified" methylene blue standard, only six were covered by a resazurin disc reading of less than 5. Of these six milks, four were rejected on the platform by organoleptic grading as being unsuitable for inclusion in the bulk. All milks showing a resazurin grade of 3 or lower were included in the reject classification. It will be observed also that all of the latter milks were included in the half-hour "modified" methylene blue group. Thus, organoleptic examination, the ten-minute resazurin test and the "modified" methylene blue test were equally efficient in detecting the grossly inferior milks.

Table 1.

COMPARISON OF TEN-MINUTE RESAZURIN TEST AND "MODIFIED" METHYLENE BLUE TEST.

Resazurin Disc Number in 10 minutes.	"Modified" Methylene Blue Test (hours).					Total Number of Samples.
	5.	4–5.	2½–3½.	1–2.	½.	
6	140	99	35	3	0	277
5	21	20	13	1	0	55
4	1	1	0	2	..	4
3	..	..	..	..	1	1
2	..	..	..	..	2	2
1	..	..	..	..	..	0
0	..	..	..	..	1	1
Total	162	120	48	6	4	340

2. Comparison with the Standard Plate Count at 37 deg. C.

The method of expression of the data in Table 2 and subsequent tables is that of Golding and Jorgensen (1945). These workers prefer the method on the grounds that the calculation of the mean and standard deviation for each resazurin grade 0 to 6 inclusive does not supply enough practical information and that a spot graph would be too indefinite. Consequently, the geometric means of the standard plate counts for each grade have been worked out and the distribution of the samples for each resazurin grade arranged on the basis of the percentage of their plate counts.

Table 2.

COMPARISON OF TEN-MINUTE RESAZURIN TEST AND STANDARD PLATE COUNT AT 37°C.

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	1	0	2	1	4	55	277
Mean Standard Plate Count (1,000 per ml.)*	25,840	..	13,360	9,440	875	135	80
Percentage of Samples with Plate Count below—							
10,000	..	..	..	..	..	..	6·1
50,000	..	..	..	..	..	25·5	40·1
100,000	..	..	..	..	25	40·0	59·9
500,000	..	..	..	..	25	83·6	87·7
1,000,000	..	..	..	..	50	90·9	92·9
10,000,000	..	..	..	100	100	100	100
50,000,000	100	..	100	..	..	..	..

* Geometric means.

An examination of Table 2 reveals a high inverse relationship between resazurin disc number and mean standard plate count. The table also shows the advantages to be gained by the rejection of certain resazurin grades. The rejection of all milks having a resazurin grade reading below 5 would imply a mean standard plate count of less than 100,000 per ml. for the accepted milk.

3. Comparison with the Direct Microscopic Count.

Though the experimental error in the compilation of Table 3 is high, especially at the right-hand end of the table where the counts are low, the overall picture is much the same as in Table 2. The rejection of all milks having a resazurin grade reading below 5 would give the accepted milk a mean direct microscopic count of less than 50,000 per ml.

Table 3.

COMPARISON OF TEN-MINUTE RESAZURIN TEST AND DIRECT MICROSCOPIC COUNT.

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	1	0	2	1	4	55	277
Mean Direct Microscopic Count (1,000 per ml.)* ..	93,940	..	9,759	4,715	268·1	39·81	38·41
Percentage of Samples with a Count below—							
50,000	..	..	..	..	..	..	78·3
100,000	..	..	..	..	..	72·7	86·3
500,000	..	..	..	..	75	85·5	96·4
1,000,000	..	..	..	..	100	100	98·9
10,000,000	..	..	50	100	..	..	100
100,000,000	100	..	100	..	..	..	..

* Arithmetic means.

4. Comparison with the Leucocyte Count.

Table 4 is open to the same criticism as Table 3. Though on the whole, high resazurin grades are associated with relatively low leucocyte counts (28.9 per cent. below 500,000, 67.1 per cent. below 1,000,000 in the case of resazurin grade 6), it has to be admitted that in the case of the better quality milks the overall picture is rather complicated owing to the lack of knowledge regarding the proportionate parts played by the bacteria and the reducing systems associated with the leucocytes in the reduction of resazurin.

Table 4.

COMPARISON OF TEN-MINUTE RESAZURIN TEST AND LEUCOCYTE COUNT.

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	1	0	2	1	4	55	277
Mean Leucocyte Count (1,000 per ml.)*	3,080	..	966	322	1,754	972	846·5
Percentage of Samples with Count below—							
100,000	..	..	..	..	..	..	1·1
500,000	..	..	50	100	..	16·4	28·9
1,000,000	..	..	50	..	25	60	67·1
10,000,000	100	..	100	..	100	100	100

* Arithmetic means.

5. Conclusions.

(*a*) This test has real value in detecting milks of very inferior quality. However, both the "modified" methylene blue test and organoleptic grading were as efficient in this respect.

(*b*) The test showed no refinement in the grading of the better class of milks as indicated by the "modified" methylene blue test.

(*c*) The test showed reasonably good correlation with the standard plate count and the direct microscopic count.

(*d*) The connection between the resazurin grade and the reducing systems associated with the leucocytes was not particularly clear in the case of the better class milks.

The One-hour Resazurin Test.

1. Comparison with the "Modified" Methylene Blue Test.

An examination of Table 5 shows that greater discrimination was obtained with the one-hour resazurin test than with the ten-minute resazurin test. Of the 340 milks examined, 154 fell in the 6-5 category, 176 in the 3-4 category and 10 in the 2-1-0 category. Acceptance of resazurin grades 4, 5 and 6 would mean the inclusion of 265 of the 282 milks giving a "modified" methylene blue test of four hours or greater. The one-hour test thus introduces greater refinement into the grading.

Table 5.

COMPARISON OF ONE-HOUR RESAZURIN TEST AND "MODIFIED" METHYLENE BLUE TEST.

Resazurin Disc Number in 1 hour.	"Modified" Methylene Blue Test (hours).					Total Number of Samples.
	5.	4–5.	2½–3½.	1–2.	½.	
6	9	1	..	..	..	10
5	82	53	9	..	..	144
4	66	54	27	..	..	147
3	8	7	11	2	1	29
2	..	2	1	4	..	7
1	..	..	..	..	..	0
0	..	..	..	..	3	3
Total	165	117	48	6	4	340

2. Comparison with the Standard Plate Count at 37 deg. C.

Table 6 shows the advantages to be gained by extending the incubation period to 60 minutes. The rejection of all grades below 5 would give the accepted milk a mean standard plate count of less than 60,000 per ml. as compared with a mean standard plate count of less than 100,000 per ml. for the ten-minute test.

Table 6.

COMPARISON OF ONE-HOUR RESAZURIN TEST AND STANDARD PLATE COUNT AT 37°C.

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	3	0	7	29	147	144	10
Mean Standard Plate Count (1,000 per ml.)*	16,640	..	1,489	284·9	103·5	69·33	18·32
Percentage of Samples with a Plate Count below—							
10,000	..	..	..	..	2·7	8·3	20
50,000	..	..	..	24·1	34·0	41·0	70
100,000	..	..	..	27·6	53·7	61·8	100
500,000	..	..	14·3	58·6	86·5	93·8	..
1,000,000	..	..	28·6	71·0	93·8	96·5	..
10,000,000	..	..	100	100	100	100	..
50,000,000	100	..	..	..	..	..	..

* Geometric means.

3. Comparison with the Direct Microscopic Count.

The rejection of all milks with a resazurin grade below 5 in Table 7 would give the accepted milk a mean direct microscopic count of less than 40,000 per ml. as compared with 50,000 per ml. for the ten-minute test.

Table 7.

COMPARISON OF ONE-HOUR RESAZURIN TEST AND DIRECT MICROSCOPIC COUNT.

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	3	0	7	29	147	144	10
Mean Direct Microscopic Count (1,000 per ml.)* ..	20,750	..	347·9	94·93	38·86	32·66	23·00
Percentage of Samples with a Count below—							
50,000	..	..	51·7	75·5	84·7	100	100
100,000	..	14·3	58·6	85·7	91·7	..	..
500,000	..	42·9	86·2	98·6	98·6	..	..
1,000,000	..	100	93·1	100	98·6	..	..
10,000,000	33·3	..	100	..	100	..	..
50,000,000	66·6	..	..	..	..	..	..
100,000,000	100	..	..	..	..	..	..

* Geometric means.

4. Comparison with the Leucocyte Count.

Though in Table 8 the highest mean leucocyte count is associated with the lowest resazurin grade reading, the relationships in the higher resazurin grades are by no means regular. This is believed to be due to the complications listed in connection with Table 4.

Table 8.

COMPARISON OF ONE-HOUR RESAZURIN TEST AND LEUCOCYTE COUNT

Resazurin Grade.	0.	1.	2.	3.	4.	5.	6.
Number of Samples ..	3	0	7	29	147	144	10
Mean Leucocyte Count (1,000 per ml.)*	1,670	..	1,110	1,211	95·1	729·8	680·8
Percentage of Samples with a Leucocyte Count below—							
100,000	..	..	..	..	·7	2·8	..
500,000	33·3	..	..	10·3	22·4	36·1	30
1,000,000	33·3	..	42·9	41·4	59·8	77·8	70
10,000,000	100	..	100	100	100	100	100

* Arithmetic means.

5. Conclusions.

(*a*) This test is more discriminating than the ten-minute test for milks tested within a few hours of production.

(*b*) Since the basic need in the bacteriological testing of milk is simplicity, it is felt that the one-hour resazurin test has no advantages to offer over the "modified" methylene blue test as a platform grading test.

(*c*) The test is reasonably well correlated with the standard plate count and the direct microscopic count.

(*d*) The relation between the resazurin grade and the leucocyte count was much the same as in the case of the ten-minute test.

GENERAL CONCLUSIONS.

It appears from the tests made that neither the ten-minute nor the one-hour resazurin test is superior to the "modified" methylene blue test for bacteriological examination of milk received at depots. Either could be employed as a correlative test, as the ten-minute test is under the British National Milk Testing and Advisory Scheme (Great Britain: Ministry of Agriculture and Fisheries, 1942.)

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A COMPARISON OF A QUATERNARY AMMONIUM COMPOUND AND A CALCIUM HYPOCHLORITE COMPOUND AS A GERMICIDAL AGENT.

By W. F. SCHUBERT, B.A., B.Sc., A.Ed., Bacteriologist, Dairy Research Laboratory, Division of Dairying.

SUMMARY.

A calcium hypochlorite compound, used at a concentration of about 50 p.p.m. available chlorine, and a di-isobutyl cresoxy ethoxy ethyl di-methyl benzyl ammonium compound, used at 200 p.p.m., were tested by the Weber-Black method with a laboratory strain of Escherichia coli as the test organism.

The hypochlorite showed the greater efficiency, probably because of the lower resistance of the gram-negative test organism.

INTRODUCTION.

Methods suggested for the evaluation of the performance of germicides fall into two classes. The first includes all tests of the phenol-coefficient-type where the germicidal value of the test substance is compared with that of a standard disinfectant. The second includes those tests which determine the rate of death of the test organism under standard conditions.

Some investigators, dissatisfied with the abstract nature of the above tests, have attempted to create in the laboratory a microcosm of the macrocosm, thereby subjecting the germicidal agents under test to as many as possible of the variables liable to be encountered under practical conditions. Johns (1947) suggested the use of glass slides on which the test organism had been dried. Mallmann and Hanes (1945) used glass rods or rings similarly treated.

Weber and Black (1948), after a careful study of existing methods, devised a procedure which involves the performance of "straight" laboratory tests with the interpretation of the results in terms of an arbitrary standard based upon the time required to sanitise heavily contaminated food utensils under practical conditions. A 100 per cent. "kill" in less than 30 seconds in the laboratory is regarded as strong presumptive evidence that "in actual food utensil sanitising procedures an exposure of two minutes would be satisfactory for ordinary non-sporing pathogenic organisms." This method was used in the experiment reported here.

MATERIALS AND METHODS.

Germicides.

Two germicides were compared, viz., (1) a quaternary ammonium compound—di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate; (2) calcium hypochlorite.

Weber-Black Method and Inhibitor.

The Weber-Black method employed is outlined hereunder:—

Five millilitres of a 2X concentration of the test germicide at 25 deg. C. are added to 5 ml. of a 2X suspension of the test organism at the same temperature as the sweep second hand of the laboratory timer reaches a pre-determined starting point. One millilitre of the test mixture is removed at exactly 15 seconds, 60 seconds, 120 seconds, and 300 seconds respectively and blown into separate 9 ml. inhibitor blanks. One millilitre and ·1 ml. of each inhibitor blank are then pipetted into petri dishes, resulting in 10–1 and 10–2 dilutions. Plates are poured with tryptone-glucose-extract medium (American Public Health Association, 1948). Initial and final plate counts of the bacterial suspension of the test organism are determined by appropriate dilutions in 99 ml. phosphate buffer dilution blanks. The pH of the test mixture is determined electrometrically.

After 300 seconds excess of the inhibitor is added to the test mixture and the walls of the tube swabbed to release any surviving bacteria. The swab is placed in a petri dish, cut with a sterile cutting instrument, and the plate poured with tryptone-glucose-extract medium. All plates are incubated for 24 hours at 35–37 deg. C.

Both lecithin and sodium oleate were used as inhibitors in separate trials with the quaternary ammonium compound in the ratio of inhibitor to quaternary of 100–1 in the 9 ml. dilution blanks. As far as could be ascertained, the inhibitors were equally effective. No information could be gained regarding the inhibitory effect, if any, of the resulting complex in either case. In the hypochlorite trials, sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was used in the preparation of the 9 ml. blanks in the concentration recommended by the American Public Health Association (1948).

Recording of Results.

After incubation the plates were counted and the results recorded in accordance with the methods of the American Public Health Association (1948) Results of the individual trials were recorded as in Table 1.

Table 1.

SAMPLE DATA RECORDING SHEET.

Germicide: Q.A.C. 200 p.p.m. Date: 22–3–50.

Escherichia coli (test strain).

5 ml. Q.A.C. 400 p.p.m.

5 ml. *E. coli* 200 x 10

pH = 7·9 at 25°C.

Exposure in Seconds.	0.	15.	30.	60.	120.	300.	Tube.	Swab
10^{0}	100 x 10^{6}	..	..	..	..	..	..	..
10^{-1}	..	0	14	1	1	0	0	T.N.C.
10^{-2}	..	0	2	0	0	1	..	..

T.N.C. = Too numerous to count.

STERILITY CONTROLS.

Agar control—0.

Water control (distilled)—0.

Dilution blank control—0.

RESULTS.

Quaternary Ammonium Compound.

Table 2 summarizes the results of 20 trials conducted with the quaternary ammonium compound using the recommended concentration of 200 p.p.m.

Calcium Hypochlorite.

Table 3 summarizes the results of 20 trials conducted with the calcium hypochlorite compound using concentrations about 50 p.p.m. available chlorine. Concentrations of available chlorine were determined by titration with N/100 sodium thiosulphate standardized against potassium permanganate (Cumming and Kaye, 1934, p. 126).

DISCUSSION.

From Table 4 it will be seen that 15 per cent. of the trials in the case of the quaternary ammonium compound and 75 per cent. in the case of the calcium hypochlorite compound gave a 100 per cent. kill within the 30 second level, which is the criterion set down by Weber and Black. The concentrations of the germicides used are those generally recommended. Quaternary ammonium compounds are generally used at a concentration of 200 p.p.m., while the Ordinance and Code Regulating Eating and Drinking Establishments of the U.S. Public Health Service recommend a minimum concentration of 50 p.p.m. available chlorine for hypochlorite compounds. The test mixture in the case of the quaternary was slightly alkaline and decidedly alkaline in the case of the hypochlorite. The concentration of the test organism in the majority of trials in both cases was about 100 million per ml.

Table 2.

SUMMARY OF QUATERNARY AMMONIUM COMPOUND TRIALS.

Experiment No.	Conc. p.p.m.	pH.	Plate Count After—										Count of Sterility Control.				
			Initial Count.	15 sec.		30 sec.		60 sec.		120 sec.		300 sec.		Tube.	Swab.	Agar.	Water.	Blank.
				10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.					
1 ..	200	7·8	103 x 10^6	5	0	10	0	1	1	1	0	0	0	4	70	0	0	0
2 ..	200	7·9	100 x 10^6	0	0	14	2	1	0	0	1	1	0	0	T.N.C.	0	0	0
3 ..	200	8·4	132 x 10^6	1	0	20	0	0	1	6	0	0	0	0	0	0	0	0
4 ..	200	8·4	78 x 10^6	11	2	14	2	30	5	21	2	3	0	0	0	0	0	0
5 ..	200	7·8	88 x 10^6	74	7	48	1	4	0	20	3	3	1	0	1	0	0	0
6 ..	200	8·0	166 x 10^6	26	3	0	0	28	4	0	0	0	1	0	T.N.C.	0	0	0
7 ..	200	7·9	176 x 10^6	44	1	32	0	0	0	12	8	2	0	0	0	0	0	0
8 ..	200	8·0	59 x 10^6	8	5	6	1	0	1	0	1	0	0	1	0	0	0	0
9 ..	200	8·4	103 x 10^6	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
10 ..	200	7·9	98 x 10^6	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0
11 ..	200	8·2	130 x 10^6	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0
12 ..	200	8·0	146 x 10^6	0	1	1	0	1	0	27	0	6	4	0	T.N.C.	0	0	0
13 ..	200	8·4	133 x 10^6	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0
14 ..	200	7·6	94 x 10^6	72	9	1	0	2	0	0	0	0	0	0	0	0	0	0
15 ..	200	7·7	91 x 10^6	7	1	1	0	1	0	0	0	0	0	0	0	0	0	0
16 ..	200	8·3	115 x 10^6	13	1	1	0	0	0	0	0	0	0	0	0	0	0	0
17 ..	200	8·3	115 x 10^6	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0
18 ..	200	7·6	102 x 10^6	15	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19 ..	200	7·6	102 x 10^6	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
20 ..	200	7·8	130 x 10^6	37	15	2	0	1	0	2	0	0	0	0	0	0	0	0

Table 3.

SUMMARY OF CALCIUM HYPOCHLORITE TRIALS.

Experiment No.	Conc. p.p.m.	pH.	Plate Count After—										Count of Sterility Control.				
			Initial Count.	15 sec.		30 sec.		60 sec.		120 sec.		300 sec.					
				10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	Tube.	Swab.	Agar.	Water.	Blank.
	p.p.m.	pH																
1 ..	53	10·8	67 x 10^6	20	0	0	0	0	0	0	0	0	0	0	2	0	0	0
2 ..	53	10·9	75 x 10^6	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0
3 ..	62	11·0	123 x 10^6	13	1	3	0	0	0	0	0	0	0	T.N.C.	8	0	0	0
4 ..	62	11·0	98 x 10^6	20	2	2	0	0	0	0	0	0	0	8	3	0	0	0
5 ..	53	11·4	120 x 10^6	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 ..	62	10·8	132 x 10^6	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
7 ..	53	11·4	78 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8 ..	53	10·9	88 x 10^6	0	1	6	2	0	1	0	0	0	0	0	1	0	0	0
9 ..	53	11·3	176 x 10^6	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
10 ..	49	12·2	98 x 10^6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
11 ..	50	10·8	134 x 10^6	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12 ..	55	11·3	165 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 ..	53	10·9	120 x 10^6	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 ..	48	11·2	130 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 ..	57	11·4	126 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 ..	57	11·4	126 x 10^6	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 ..	57	11·2	126 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 ..	60	11·3	108 x 10^6	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
19 ..	60	11·3	108 x 10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 ..	60	11·4	108 x 10^6	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.

ANALYSIS OF RESULTS.

Germicide.	Conc. p.p.m.	pH Range. 25°C.	Range of Initial Concentration.		100% Kill Within.				
			Max.	Min.	15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
Quaternary Ammonium Compound	200	7·6–8·4	130×10^6	59×10^6	10	15	30	50	60
Calcium Hypochlorite ..	48—62	10·8–11·4	176×10^6	67×10^6	55	75	80	100	..

The greater efficiency of the hypochlorite may in fact be due to the lower resistance of the gram-negative test organism to the hypochlorite than to the quaternary ammonium compound. Since the gram-negative organisms are the chief quality affecting types in the dairying industry, hypochlorites would thus appear to possess an intrinsic advantage over the quaternary ammonium compounds for dairy sanitation.

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